A METHOD OF STAINING THIN SECTIONS WITH LEAD HYDROXIDE FOR PRECIPITATE-FREE SECTIONS

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Staining of thin sections results in greater contrast which facilitates focusing and the observation of fine structure. Lead hydroxide, as suggested by Watson (1), has become increasingly popular as a stain for electron microscopy. However, when lead hydroxide is exposed to the carbon dioxide of air, lead carbonate forms and is deposited on the sections as fine, needle-like crystals, large amorphous or polygonal precipitates, or small granules. In order to prevent the stain from coming in contact with air, Peachey (2) used a plastic, air-free syringe as a receptacle for the stain and as a mechanism for staining sections. The cover of the syringe was filled with a carbon dioxide trap consisting of sodium hydroxide, anhydrous calcium chloride, and cotton. Parsons and Darden (3) designed a

staining apparatus with which six grids could be stained at once without the danger of precipitate formation. A more complicated mechanism which serves as a barrier between the lead hydroxide stain and air was built by Huxley and Zubay (4).

Other investigators proposed variations in stain preparations and staining procedures designed to discourage contamination. Dalton and Zeigel (5), after staining sections with lead acetate, exposed them to ammonium hydroxide vapors; thus, the formation of lead hydroxide occurred in the sections themselves. Millonig (6) added tartrate to the lead hydroxide solution to stabilize the lead salt so that protection from air was not necessary. Highly alkaline lead stains were found by Karnovsky (7) to be more stable than those of a lower pH. Lever (8) suggested cleaning the stained sections by dipping them in 1 per cent potassium hydroxide.

The staining procedure described here is a simple means of preventing any extensive stain-air contact since this will cause the formation of precipitate in the staining solution and consequently on the sections. The method is reproducible and has consistently resulted in precipitate-free sections.

PREPARATION OF THE STAIN

A 1 per cent solution of lead hydroxide is prepared by boiling distilled water for about 5 minutes, removing the water from the hot plate or Bunsen burner, and adding the lead hydroxide powder to the water. The only available source of lead hydroxide found by the author is the Amend Drug and Chemical Company, New York City. The solution is stirred with a glass rod for 1 to 2 minutes; it is then filtered through two sheets of No. 12 Whatman filter paper into 12- to 15-ml glass centrifuge tubes. Each tube is filled with about 10 ml of solution which now should appear clear. However, because of variation in batches of lead hydroxide, the filtered solution might, in some instances, be slightly cloudy. If this occurs, one drop, or two if necessary (a disposable pipette is recommended), of 40 per cent sodium hydroxide in distilled water[1] should be added to each tube to clarify the solution. About one-quarter inch of mineral oil is poured into each tube to protect the stain from contact with air. The tubes are sealed with rubber stoppers and the stain can be stored as such for 3 to 4 months.

STAINING PROCEDURE

The staining vessel consists of a series of wells, large enough to hold two grids each, drilled in a plastic (Lucite) bar.[2] The bar employed by the author is 8 inches long, ¾ inch wide, and ½ inch thick. It contains thirteen wells, each ¼ inch in diameter. If a bar containing wells is not available, one or more beakers, about one-half inch or less in diameter, may be substituted. A disposable pipette is used to draw up the stain and is wiped free of mineral oil with lens tissue. The first one or two drops of stain from the pipette are discarded. One or two wells at a time are filled with sufficient stain to produce a convex meniscus. The grid or grids are placed at the bottom of the wells filled with stain. Coverslips, which had previously been cleaned with lens tissue, are immediately placed over the wells. The meniscus of the stain should be sufficiently high to prevent the formation of air bubbles when the coverslips are positioned over the wells. During the entire procedure, the bar is kept resting in an enamel pan, 8½ inches long, 3¾ inches wide, and 1¾ inches deep. A glass plate, 8 inches long, 1½ inches wide, and ¼ inch thick, is placed over the bar.[3] Staining time for Epon-embedded sections on uncoated grids is from 12 to 15 minutes. Coated grids may require about 30 minutes since the stain penetrates from one side only. Methacrylate sections which stain more readily may need only about 5 minutes. When the sections have been stained for the necessary length of time, the pan is filled with distilled water that has boiled in a covered flask for at least 15 minutes, and cooled sufficiently to insure comfortable handling. Cooling of the water may be expedited by running cold water along the sides of the flask for 5 to 10 minutes. The surface of the water in the pan is wiped with lens tissue. The glass plate and coverslips are gently moved off the bar with a pair of clean forceps. After agitating the water in the pan to facilitate mixing with and dilution of the stain within the wells, the water is partially replaced with additional water from the flask. The water surface of the pan is wiped again with lens tissue and the

[1] The author is indebted to Dr. Giuseppe Millonig for the suggestion to use 1 per cent lead hydroxide and 40 per cent sodium hydroxide.

[2] The author would like to thank Theodore Nadeje of The Rockefeller Institute for the plastic bar.

[3] The glass plate prevents the coverslips from becoming dislodged while the pan is being filled with water.

bar removed from the pan. The individual grids are then briefly rinsed in water from the flask and placed on filter paper.

If a bar containing thirteen wells is used, twenty-six grids may be stained simultaneously. The entire procedure requires only about one hour and regularly yields sections free of contamination. The resulting contrast of the sections is essentially the same as that obtained by Watson (1) and Millonig (6).

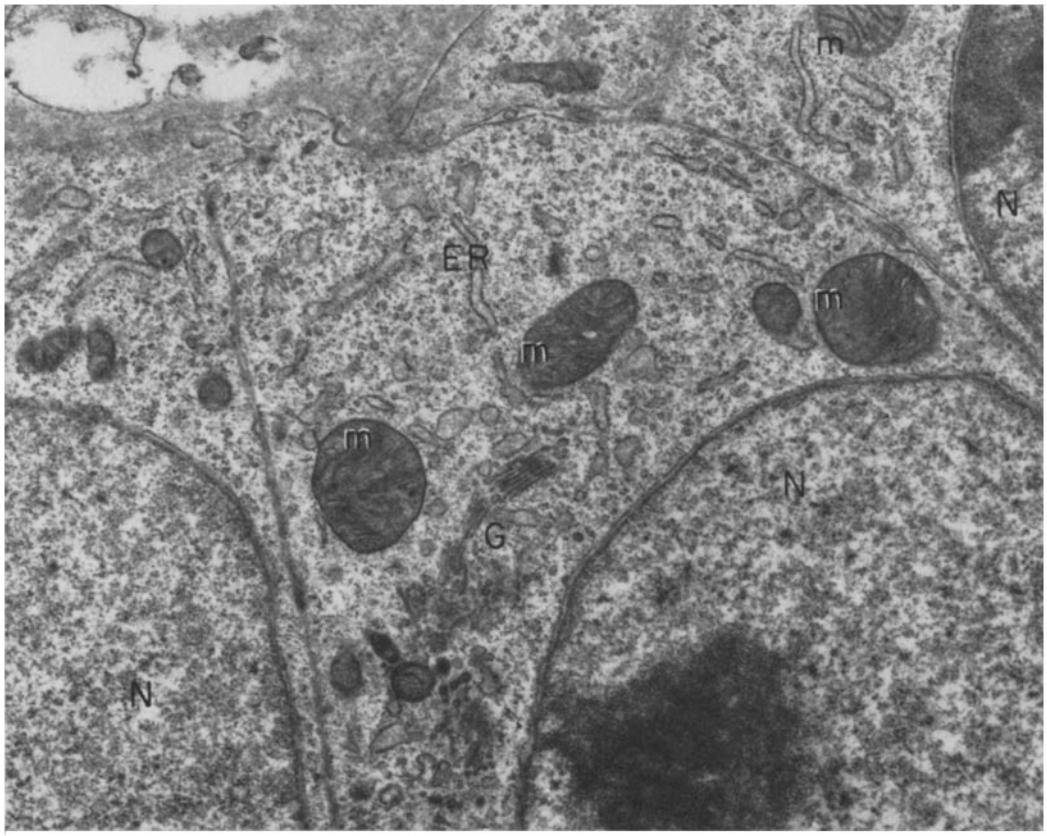

FIGURE 1

Section of a mouse mammary tumor. Fixed in osmium tetroxide; embedded in Epon; sections placed on uncoated grids and stained with lead hydroxide, employing the technique described. *N*, nucleus; *m*, mitochondria; *G*, Golgi apparatus; *ER*, endoplasmic reticulum. × 16,000.

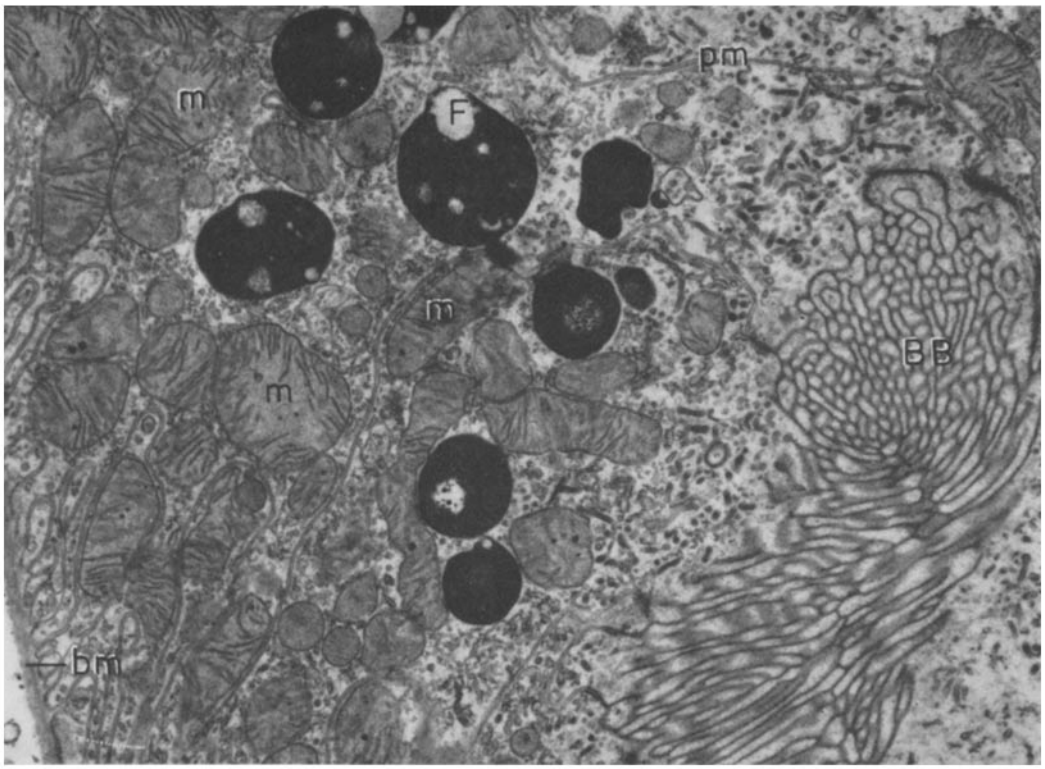

FIGURE 2

Section of proximal convoluted tubule of mouse kidney. Fixed in osmium tetroxide; embedded in Epon; sections placed on uncoated grids and stained with lead hydroxide, employing the technique described. *BB*, brush border; *m*, mitochondria; *F*, fat droplets; *pm*, plasma membrane; *bm*, basement membrane. × 16,000.

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